REMARKS

The following discussion and new evidence are submitted in response to the arguments in the Examiner's Answer. The headings below refer to the rejections made in the Final Rejection and repeated in the Examiner's Answer.

Rejection Under 35 U.S.C. 112 (First Paragraph)

Claims 1-7 and 10 have been rejected under 35 U.S.C. 112, first paragraph as it is argued that they do not comply with the written description requirement. Applicants respectfully disagree.

It is argued, at page 3 of the Examiner's Answer, that the phrases "elution chromotagraphy" and "whereby suitable elution chromatography parameters for separating the biological sample into biomolecules are ascertained" are not described in the specification. This is submitted to be incorrect. At page 3 of the Examiner's Answer, the Applicant's arguments are characterized as relying on the use of the term "eluate" in the specification as teaching elution chromatography. This is but one part of the greater whole which teaches that the present specification employs elution chromatography. While it is true that displacement chromatography also uses an eluent, it is clear from the specification and examples in the present application that elution chromatography is taught. It is again noted that the Federal Circuit's predecessor court has established that it is not necessary to find an exact phrase in the claims somewhere in the specification in order to satisfy the written description requirement. See *In re Wertheim et al.*, 541 F2d. 257, 191 U.S.P.Q. 90 (CCPA 1976). Instead, the standard for written description is whether the specification teaches one of ordinary skill in the art that the inventors invented the invention of the claims. The present specification clearly teaches the concept of elution chromatography, and of determining suitable elution chromatography parameters. For example, at page 4, lines 1-20 in the specification, it is stated that the invention is "suitable for the automated search for suitable chromatography media and the associated buffer *and elution conditions* for the purification of peptides and proteins, but also other bio-molecules." The noted

portion of the specification continues that the "bio-molecules absorbed onto the gel particles are re-dissolved from the gel particles by means of a suitable elution solution (mobile phase)." The passage concludes that, "it is possible to determine a wide variety of chromatography parameters, such as, for example, gel media, buffers, pH, solvent additives or substances for the stabilization of bio-molecules." Other portions of the specification also make it amply clear that the inventions is directed to the determination of elution parameters:

line 4 of the abstract: "solution for desorption (elution) of the bio-molecules bound to the gels."

line 6 of the abstract: "of the unbound bio-molecules and/or the eluates."

page 3, line 26 of the specification: "Of such solutions and substances (henceforth referred to as elution solutions) is the ability for desorption (elution) of the biological sample from the chromatography gel,"

page 3, line 29: "that the bio-molecules bound to the chromatography gel have no or only low affinities to the chromatography gel after addition of the elution solution."

page 5, line 5: "To this end, the multiwell plates may already be prefabricated with chromatography media for binding the biological sample (group B materials and a set of very different chromatography media (solutions of any composition) for elution (group NB materials)."

page 5, line 12: "media distributed on multiwell plates, and eluents matched to the respective chromatography media…"

page 6, line 3: "suitable chromatography media and the associate eluents in a very short time…"

page 8, line 1: "in the eluates of the multiparallel chromatography system."

See also:

page 10, line 4,

page 10, line 20,

page 11, lines 6 and 27-31,

page 13, lines 13-16,

page 15, line 6,

page 16, lines 9-12, 20-22 and 31,

page 17, lines 22-23,

page 18, line 9-11,

page 19, line 9-11

and especially the examples, which would be recognized by one of ordinary skill as detailing the conducting of elution chromatography.

Accordingly, it is submitted that the specification and examples clearly support the claims.

Rejections Under 35 U.S.C. 102 and 103

Claims 1-7 and 10 have been rejected under 35 U.S.C. 102(b), or in the alternative 103, over Cramer '086. It is argued, for example, at page 4 of the Examiner's Answer, that parameters determined would be equally applicable to both elution chromatography and displacement chromatography (as in Cramer, which discloses only displacement chromatography). It is thus concluded that the determination of parameters as in the present claims would be of the same parameters determined to conduct displacement chromatography. Applicants respectfully disagree with this analysis. First, attention is directed to the article of Dr. Little, "Displacement Chromatography Becomes of Age." The article discusses the differences between elution chromatography and displacement chromatography, and the parameters which are determined for operating each. For example, Little discloses that "limitations of elution mode chromatography are virtually unknown in displacement mode chromatography, where isocratic flow of mobile phase and high column loadings are the rule." Little indicates that displacement chromatography does not employ gradients, does not employ salts which must be removed at some point, operates on standard stationary phases instead of special chromatography media and, as the Examiner notes in the Examiner's answer, employs a displacer.

In addition, attention is directed to the attached Wikipedia article on displacement chromatography, which discusses the "distinct differences" between displacement and elution chromatography. The article notes that elution chromatography employs gradient solvent pumping and low through put, but instead in displacement chromatography components are

resolved into consecutive zones rather than peaks. It is thus clear that optimizing parameters (e.g., media, solvates, salts, etc.) for elution chromatography is simply not the same as optimizing the very different parameters employed in displacement chromatography.

It is thus clear that the disclosure of displacement chromatography in Cramer does not teach one of ordinary skill in the art to conduct the process of the present claims in order to determine the relevant parameters for elution chromatography. In contrast to the teaching of Cramer, the matter of the present invention is based on methods of elution chromatography and has as a goal developing a simple method ascertaining (with minimal effort) optimized conditions for purifying biological samples, preferably for the separation of biological molecules, using elution chromatography. This means that, in each case, the biological samples are bound to the chromatography media. Then materials (solutions and substances) are added, which are able to desorb (to elute) the biological sample from the chromatography medium in a way as described on page 3, last paragraph of the present specification. The biological sample is removed from the chromatography material but is not displaced by a further compound, unlike the situation with displacement chromatography as in Cramer.

Because the present invention involves a method for the purification of biological samples and isolation of biological molecules on a large scale, the chosen chromatography material has to be useable continuously for the purification or separation in an ongoing process. Therefore, conditions of displacement chromatography would not be suitable. Thus, Cramer, which clearly distinguishes elution chromatography from their method, does not anticipate the present claims, nor suggest them. One of ordinary skill in the art would not transfer any teachings from the displacement method of Cramer to elution chromatography, in view of the above discussed substantial differences in materials, technique and conditions.

By way of further explanation, the divergent techniques can be explained as follows.

There are three different types of liquid chromatography: 1) isocratic elution, 2) elution chromatography and 3) displacement chromatography. These types of chromatography are each carried out in entirely a different technical manner; moreover the basic physicochemistry which leads to separation is different. In the case of isocratic elution the compounds to be separated are divided from one another according to their affinities for a stationary phase and for the mobile

phase. Molecules with high affinity for the stationary phase move with less speed across the column than molecules with low affinity, because the probability of residence of molecules with high affinity for the stationary phase is higher than that for molecules which stay in the mobile phase. In consequence, the molecules are eluted according to a Gaussian bell curve.

The separation mechanisms in elution chromatography are the same as described for the isocratic elution of compounds, but with the difference that during the course of separation a second mobile phase is added. This secondary mobile phase speeds up the elution of molecules from the stationary phase by weakening or preventing the interaction between the molecules and the stationary phase. This effect is also achieved if the second mobile phase is added in big excess. Molecules of the second phase in excess shield the sample molecules, which shall be separated, from the interaction with the stationary phase. The effect is a decrease of the probability of residence of molecules with high affinity for the stationary phase and a decrease of affinity for the stationary phase.

On the other hand, in separation carried out by means of displacement chromatography, the affinity for the stationary phase stays the same during the whole separation procedure, because the separation is carried out with only one mobile phase. In displacement chromatography, a molecule with high affinity for the stationary phase or chromatography matrix (the displacer) competes effectively for bonding sites, and thus displaces all molecules with lesser affinities for the stationary phase. In order to elute the molecules, which interact with the stationary phase, a second molecule is added to the eluent in low concentration. This second molecule displaces the desired molecule from the stationary phase. The desired molecules cannot return to the bonding sites if the displacer molecules follow directly. This means, that molecules with higher affinity displace molecules with less affinity for the stationary phase.

In contrast to the mechanism of elution chromatography, in displacement chromatography the desired molecules of the sample and the displacer molecules compete for the bonding sites of the stationary phase. In order to achieve a good separation result the flow rates during displacement chromatography are reduced to a tenth of the separation techniques described above. Because of the separation mechanism during displacement chromatography the affinity for the stationary phase plays a minor role, unlike elution chromatography, where affinity for the

stationary phase dominates.

The disclosure of Cramer deals with the physicochemical laws of displacement chromatography, but disregards the principles of elution chromatography. According to the teachings of Cramer, the skilled artisan is able to find suitable displacers, but gets no information allowing selection of suitable parameters for elution chromatography, as claimed in the present application, e.g., the second mobile phase, the chromatography media, etc., all of which have been explained in detail above to differ between chromatography types. Therefore, the teaching of the present application is in no way an "optimization" of the steps of Cramer, but a complete departure therefrom, with respect to the parameters which are determined. Thus, even if it would be "obvious to optimize" the separation of Cramer, doing so would not result in optimizing *elution* conditions, which are very different from those employed in displacement chromatography. Accordingly, withdrawal of this rejection is respectfully requested.

Claims 1-7 and 10 have also been rejected under 35 U.S.C. 103 over Cramer taken with Welch '160. Welch also does not suggest the use of elution chromatography. Welch discloses separation of an enantiomeric pharmaceutical active substance (Naproxen®) and the screening for finding suitable adsorbents. As a result, a combination of Cramer and Welch would teach to one of ordinary skill in the art a method involving identification of a suitable adsorbent and of suitable displacer molecules for the adsorbed molecule to be isolated. This clearly does not suggest determination of parameters for a method for the separation and purification of bio-molecules as presently claimed, using elution chromatography. Withdrawal of this rejection is therefore also appropriate, and respectfully requested.

Claim 5 has also been rejected under 35 U.S.C. over Cramer taken with Welch, further in view of MacPhee '753, Snyder '242 and Pantoliano '293. This rejection has essentially been discussed previously. The secondary references are cited primarily for their disclosure of stabilization of proteins with glycerol. Regardless, it is submitted that these references do nothing to remedy the deficiencies of the primary reference, in that elution chromatography parameters are not suggested to be ascertained by the method of the primary reference. There is simply nothing in the secondary references which would teach expanding the method of the primary reference from displacement chromatography to elution chromatography. Moreover,

MacPhee discloses a large number of possible stabilizers. Glycerol is one substance of the disclosed group and is not preferred. A very large list of possible stabilizers is given by MacPhee in U.S. 2003/0161753 A1 on page 4, in column 2 up to page 5, column 1, with divergent moieties listed: sugars, enzymes, fatty acids, amino acids, vitamins and so on. Furthermore, the stabilizers named by MacPhee are compounds that alone or in combination reduce damage to the biological material being irradiated to a level that is insufficient to preclude the safe and effective use of the material. This reference accordingly does not teach the use of glycerol to stabilize biomolecules in a chromatography process.

The Commissioner is hereby authorized to charge any fees associated with this response or credit any overpayment to Deposit Account No. 13-3402.

Respectfully submitted,

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Attorney Docket No.: MERCK-3002

Date: June 22, 2009